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(54) Title: ANTISENSE TREATMENT OF PULMONARY HYPERTENSION

(57) Abstract

The invention herein described relates to a method to treat pulmonary hypertension by antisense therapy using ET–1 derived antisense molecules delivered to the lungs as a pulse/spike in an inhaler.

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ANTISENSE TREATMENT OF PULMONARY HYPERTENSION

The invention relates to a method and means to treat, but not exclusively, pulmonary hypertension by functionally suppressing the product of the ET-1 gene which encodes a vasoconstrictor peptide the expression of which is correlated mainly with this disorder.

Pulmonary Hypertension (PH) is a disorder in which the blood pressure in the pulmonary (lung) arteries is abnormally high in the absence of other diseases of the heart or lungs. PH is considered to be present when the mean pulmonary arterial pressure is greater than 25mmHg at rest or 30mmHg during activity. The normal mean pulmonary – artery pressure is approximately 14mmHg at rest. PH is classified according to whether the disorder is primary or secondary PH.

Primary PH (PPH) is a relatively rare disorder affecting mainly women between the ages of 21 and 40 although men and children can suffer from the disease. The incidence annual rate is as high as 2 per million of the UK population. In the USA approximately 300 new cases of PPH are diagnosed each year. The exact cause of PPH is unknown, this is mainly due to the low incidence of PPH and the lack of an effective animal model in which to study the disease. However PPH is associated with cirrhosis, AIDS and can be familial.

Secondary Pulmonary Hypertension (SPH) is present where there is an identifiable cause resulting in an increase in pulmonary arterial pressure. These include left venticular failure, chronic hypoxia, lung disease or alveolar hypoventilation and a number of systemic diseases such as lupus

erythromatosis, systemic sclerosis, SPH may also result from chronic thrombo-anabolic disease.

For both PPH and SPH the symptoms are similar. These include breathlessness, sycope (faints), angina (chest pain), headaches, fatigue, blurred vision, insomnia, and intestinal complaints. SPH is much more common and is a major killer in the Western world.

There are currently a number of drug treatments that can help to ameliorate pulmonary hypertension. The successful clinical treatments include the continuous intravenous administration of prostacyclin (PGI_2) and inhaled nitric oxide (NO), for severe disease; for milder degrees of illness, anticoagulants and calcium channel blockers can be used to lessen symptoms and improve survival.

In pulmonary hypertension, sufferers respond differently to different drugs therefore no single drug regime can be used to blanket treat sufferers. Moreover dosages have to be empirically decided due to the potential for adverse side effects. For example, systemic low blood pressure, nausea, angina, headaches and flushing. Therefore alternative strategies are required to provide therapeutic treatment of pulmonary hypertension.

The product of the gene endothelin-1 (ET-1) has been shown to act as a potent vasoconstrictor and is involved in the remodelling of blood vessels of the lung to cause narrowing and obstruction. The product of the ET-1 gene is found in endothelial cells that line arteries and has been shown to be elevated in expression in individuals and in animal models that show pulmonary hypertension. Moreover, current successful treatments of clinical and experimental and hypertension using PGI_2, and NO have been shown to

inhibit the expression of ET-1. This correlative data is strongly suggestive that ET-1 is an important component in mediating pulmonary hypertension.

Indeed, the drug BQ-123 has been shown to antagonise the activity of ET-1 receptors and relieve pulmonary hypertension resulting from congestive heart failure. In rat models, mRNA to ET-1 was shown to decrease as a consequence of long term BQ-123 treatment.

The problems of drug side effects, amongst other things, has lead to alternative therapeutic strategies to be devised. A considerable effort is being made toward developing gene therapy approaches to control disease pathogenesis and it is likely that in subsequent years this will provide a serious alternative to the use of pharmaceuticals.

A recent strategy to interfere with the expression of a gene is that of antisense technology. In brief this strategy involves the use of a DNA or RNA molecule that is complementary to a region of a selected gene and is able to hybridise (bind) under physiological conditions to the targeted nucleic acid to prevent either transcription of the gene or translation of the mRNA encoded by the gene. The antisense molecule is often a short oligodeoxynucleotide (ODN). However the molecule may be an oligodeoxyribonucleotide, or a modified oligodeoxynucleotide, or a modified oligodeoxyribonucleotide; each of which are able to hybridised to a selected part of a gene, or mRNA, under physiological conditions. The modifications to oligodeoxynucleotides will be apparent to one skilled in the art. The exact region of the nucleotide sequence of the gene to which the antisense molecule is designed can be empirically determined. However it is common practice to design oligodeoxynucleotides to the 5' region of the gene (to interfere with transcription initiation) or the mRNA (to interfere

with translation). As stated before, the region of the gene to which the antisense molecule is directed is determined by the efficiency with which the antisense molecule suppresses the gene of interest. Contrary to the above this may be the 3' region and is determined experimentally. The length of the ODN also has to be determined experimentally. Typically ODNs are 20-30 nucleotides in length but may be much longer.

Presently, a number of delivery methods are being explored for their effectiveness at delivering gene therapy to a specific tissue. Viral vectors and liposomes have been used with some success. Recently, cationic lipids chelated to a therapeutic DNA molecule have been shown to deliver the DNA molecule to the lungs (1). Cationic lipids are positively charged lipids which when combined with negatively charged DNA form a tight complex. Moreover, the lungs are coated in a surfactant, a major component of which is dipalmitoylphosphotidylcholine, which is cationic at physiological pH and which is internalised by endothelial cells that line the arterioles of the lungs.

In our co-pending application (GB9805185.7 ref. 2) the delivery of an aerosol containing a vasodilator by a specialised inhaler is described. As an alternative to the continuous perfusion of the lungs using a nebuliser, the therapeutic composition, ie aerosols, is delivered intermittently and in short pulses using the so called "spike principle". By using this methodology the medicament is driven deep into the lung tissue only during inhalation. There are many lung diseases and conditions suffered by humans for which the preferred form of treatment involves delivering a medicament of the appropriate sort down a patient's airways into the lungs, where the medicament can act upon, and perhaps be absorbed into, the tissues of the lungs. The most effective treatments for these disorders involve the inhalation of some suitable chemical agent in inhaled air. The co-pending

application describes the administration of nitric oxide, a known vasodilator, in the form of a pulse or "spike". In the treatment of the constriction of the small pulmonary arteries, the very short pulse of nitric oxide is provided at the start of the inhalation, such that the resultant bolus of nitric oxide mixture inhaled by the patient has a nitric oxide concentration high enough to have the desired therapeutic effect, even if admixed with some additional air, but is of such short duration (both in time and as a result, in physical amount) that, pushed by the following much larger volume of plain, and therefore nitric oxide – free, air/oxygen, it reaches deeper into the lungs, where it both acts on the small pulmonary arteries and is taken up into the capillaries,

The invention therein described is a small (pocket-sized) hand held treatment apparatus that utilises the "spike" principle. The invention comprises a conventional nebuliser (having a reservoir in which is stored the medicament to be administered) driven by some suitable pressurised gas from a valved cylinder so as to deliver a medicament into a tube through which the patient is breathing (by mouth) normal air, the gas cylinder valve being controlled by a suitably programmed computing device that is fed data describing the pressure within the breathing tube and so is able to open and close the valve at, and for, a time such as to drive the nebuliser to deliver (to the tube and thence to the patient's lungs) a required pulse of medicament at any selected point within the patient's respiratory cycle. The idea of this is simply to ensure that a high concentration of the medicament reaches and affects the target area, and the target area alone, rather than having the whole of the lungs subjected to it.

The apparatus of the invention incorporates a nebuliser in the reservoir of which is storable the medicament to be administered. The reservoir has a fine orifice through which its contents may exit and across which is blown a

stream of gas along a pipe way. As the gas flows past the orifice it causes an external pressure drop (relative to the reservoir's internal pressure) resulting in some of the reservoir's contents being sucked out into the gas stream creating an aerosol-like cloud of fine drops or particles that is swept along the pipe way by the driving gas. The driving gas is contained within a valved cylinder, the operation of the valve being controlled by a computing means in response to the input from a pressure sensor. The pressure sensor is located within the breathing tube and provides the computing means with data about the air pressure there within, and thus about the patient's respiratory cycle.

The invention therefore comprises a nebuliser with a reservoir containing a therapeutic composition which has contact with a gaseous supply through which the therapeutic composition is aerosolised. The delivery of the composition via the breathing tube is regulated by a valved cylinder which operates according to pressure data received from a pressure sensing means located in the breathing tube to determine the stage of the patient's respiratory cycle. The whole system is computer controlled to ensure that the pulse of therapeutic composition is delivered at the appropriate stage of the patient's respiratory cycle.

We have used an inhaler employing the above 'spike' methodology and in one example the specialised inhaler described in GB9805185.7 to create an aerosol of ET-1 antisense molecules to perfuse lung tissue to relieve pulmonary hypertension. The designed antisense ET-1 molecules will target either the ET-1 gene and/or the ET-1 mRNA to suppress the ET-1 gene product from the endothelial cells that line the lung arterioles and thereby relieve pulmonary hypertension.

It is therefore an object of the invention to design antisense ODNs to ET-1 for use in the alleviation of pulmonary hypertension.

It is yet a further object of the invention to deliver antisense ODNs to the lungs to alleviate pulmonary hypertension.

According to a first aspect of the invention there is provided a method to treat pulmonary hypertension comprising administering a therapeutic composition to an individual, wherein said composition comprises at least one aerosolised antisense ET-1 molecule, delivered to the lungs by an inhaler during inhalation by a patient.

Ideally said antisense material is of mammalian origin, and most ideally of human origin.

It will be apparent from an analysis of the prior art that the biological effects of ET-1 are mediated through the receptors ET-A and ET-B. Therefore although embodiments of the invention will be described that target ET-1 with antisense ET-1 molecules it will be apparent that targeting ET-A and/or ET-B may achieve the desired result of suppressing ET-1 activity although not directly. Therefore the following description assumes that one skilled in the art would appreciate that the antisense strategy could be applied to suppress ET-A and/or ET-B activity.

According to a further aspect of the invention there is provided a method to treat pulmonary hypertension comprising delivering aerosolised antisense ET-1, or ET-A or ET-B molecules to the lungs by an inhaler as a pulse/spike during inhalation.

According to yet a further aspect of the method there is provided a therapeutic composition for treating pulmonary hypertension comprising; the non-coding strand of the ET-1 gene, or fragment thereof, as represented in Figure 1.

It will be apparent from Figure 1 that the sequence presented is the sense strand of the ET-1 gene. Antisense molecules would be designed to represent sequence complementary to this sequence and can be easily deduced from the sense sequence.

In a preferred method of the invention said antisense ET-1 molecule is a oligodeoxynucleotide, a modified oligodeoyxnucleotide, an oligodeoxyribonucleotide or a modified oligodeoxyribonucleotide represented by the DNA sequence shown in Figure 1, that is the non-coding strand of ET-1.

Reference herein to modifications is intended to include without limitation modifications apparent to those skilled in the art that improve stability (both as a stored composition and during *in vivo* use; improve permeability across biomembranes or facilitate aerosolisation).

In a preferred method of the invention the antisense molecule is between 10-100 nucleotides in length.

In yet a further preferred method of the invention said antisense molecule is greater than 100 nucleotides in length.

In yet still a further preferred method of the invention said oligonucleotide has a G + C content of at least 50% and more preferably still a G + C content of between 50% and 60%.

More preferably still said oligonucleotides do not contain a consecutive run of more than four bases of any one kind.

In yet still a further preferred embodiment of the invention said oligonucleotide has a predicted melting temperature of at least 55^0, and more preferably still, a melting temperature of between $55^0 - 80^0$.

In a preferred embodiment of the invention there is provided at least one human ET-1 antisense oligonucleotides as represented in Table 1.

Table 1

HsET117	CAG CCC AAG TGC CCT TTA AC
HsET132	CTC AAA GCG ATC CTT CAG CC
HsET318	AGC TCA GCG CCT AAG ACT GC
HsET438	TGG CAG AAG TAG ACA CAC TC
HsET779	TGG TCT CCG ACC TGG TTT GT
HsET868	ATG TGC TCG GTT GTG GGT CA

In yet a further preferred embodiment of the invention there is provided at least one rat ET-1 antisense oligonucleotides as represented in Table 2.

Table 2

RnET198	ACA GCA GAG AGA AGA TCA CG

RnET294	TGC ACT TCC TTC TCA GCT CG
RnET466	GGA TCG CTT AGA CCT AGA AG
RnET718	CTT GAT GCT GTT GCT GAT GG
RnET795	AGT CAA TGT GCT CGG TTG TG
RnET1081	ACT GTG TCT CTG CTC TCC GA

It will be evident to one skilled in the art, and from the above description, that a reliable animal model to test the efficacy of therapeutic treatments for pulmonary hypertension currently does not exist. It would therefore be desirable to develop an animal model system to test the efficacy of antisense ET-1 molecules. The sequences identified in table 2 represent preferred rat sequences. However it will be apparent to one skilled in the art that oligonucleotides derived from other species may also be useful in practising this method of the invention. Particularly those sequences that have a high degree of sequence homology to those sequences presented in Tables 1 and/or 2.

According to a further aspect of the invention there is provided a method for determining the efficacy of antisense ET-1 molecules comprising exposing a known hypersensitive animal model system to antisense ET-1 for studying molecules and observing the effects of same on pulmonary hypertension.

In a preferred embodiment of the invention said therapeutic treatment is based on antisense therapy. Ideally said antisense therapy is based on the ET-1 sequence and more preferably still those sequences represented in Table 1 and/or Table 2, and/or sequences homologous or analogous thereto,

wherein said homology is at least 50%, ideally 75% and preferably at least 90%.

In yet a further preferred method of the invention there is provided a modified antisense ET-1 molecule as herein described for the treatment of pulmonary hypertension.

It will be apparent to one skilled in the art that a number of potential modifications can be made to the antisense oligodeoxynucleotide molecules to improve efficacy and/or stability of said molecules. These include modifications to the phosphodiester bonds between nucleotides, (eg the inclusion of peptide bonds to form peptide nucleic acids). Other modifications may be to the bases and/or sugars by the covalent attachment of chemical groups to specific sites in the sugar and/or base. All of these modifications will not affect the binding properties of the antisense molecule to its target site in ET-1.

According to a further aspect of the method there is provided an antisense molecule adapted to hybridise to the transcriptional promoter region of the ET-1 gene to inhibit transcription of said gene.

It will be apparent from an analysis of the prior art that the biological effects of ET-1 are mediated through the receptors ET-A and ET-B. Therefore although embodiments of the invention will be described that target ET-1 with antisense ET-1 molecules it will be apparent that targeting ET-A and/or ET-B may achieve the desired result of suppressing ET-1 activity although not directly. Therefore the following description assumes that one skilled in the art would appreciate that the antisense strategy could be applied to suppress ET-A and/or ET-B activity.

It will be apparent to one skilled in the art that the antisense ET-1 molecule used in this way can be designed to either the sense strand or antisense strand of the ET-1 transcriptional promoter as the target sequence is double stranded genomic DNA.

According to yet a further aspect of the invention there is provided an antisense molecule adapted to hybridise to splice junctions between exons and introns of prepromessenger RNA encoding the ET-1 protein.

It will be apparent to one skilled in the art that the expression of the ET-1 gene can be inhibited by interfering with the splicing of introns from the pre mRNA of ET-1 to prevent the formation of a mature, translatable mRNA.

According to a further aspect of the invention there is provided an inhaler comprising means for administering the aforedescribed antisense molecules encoding ET-1 nucleic acid wherein said administration is via a spike or pulse and preferably only during inhalation by the patient.

According to yet a further aspect or embodiment of the invention said inhaler is disposable and adapted to receive a cartridge containing antisense molecules encoding the ET-1 nucleic acid composition.

According to yet a further aspect of the invention there is provided a method for administering the composition of the invention comprising;

i) adding or providing the antisense molecular composition in a suitable diluant, carrier or excipient and in dosage form to, or in a reservoir of, an inhaler;

ii) aerosolising said composition by a suitable means;

iii) detecting the air pressure within the inhaler by a suitable sensing device to determine a patient's respiratory cycle; and

iv) delivering a prescribed dose of the afore described therapeutic composition to the patient's lungs during a selected inhalation phase of the patient's respiratory cycle.

In yet a further aspect of the method there is provided a kit used to treat pulmonary hypertension comprising; a therapeutic antisense composition as hereindescribed; an inhaler, including a nebuliser, a reservoir for containment of said composition, an air pressure sensing device and a valve containing pressurised cylinder means to regulate the dosage of said composition.

The invention hereindescribed therefore provides a means of controlling pulmonary hypertension using aerosolised antisense molecules to the ET-1 gene, and/or mRNA, to inhibit the vasoconstrictor activity of the ET-1 peptide. Moreover, the delivery of the antisense molecule makes use of an inhaler that functions during patient inhalation to drive the therapeutic composition deep into the lungs to deliver the antisense molecule to the endothelial cells that line the arterioles of the lungs and thereby target those blood vessels that result in pulmonary hypertension.

An embodiment of the invention will now be described, by example only, and with reference to the following Figure and methods wherein;

Figure 1 represents the genomic DNA sequence of human ET-1.

Antisense Design Protocol

Table 1

Human sequences

HsET117	CAG CCC AAG TGC CCT TTA AC
HsET132	CTC AAA GCG ATC CTT CAG CC
HsET318	AGC TCA GCG CCT AAG ACT GC
HsET438	TGG CAG AAG TAG ACA CAC TC
HsET454	CCA AAT GAT GTC CAG GTG GC
HsET779	TGG TCT CCG ACC TGG TTT GT
HsET868	ATG TGC TCG GTT GTG GGT CA

Table 2

Rat sequences

RnET198	ACA GCA GAG AGA AGA TCA CG
RnET294	TGC ACT TCC TTC TCA GCT CG
RnET466	GGA TCG CTT AGA CCT AGA AG
RnET718	CTT GAT GCT GTT GCT GAT GG
RnET795	AGT CAA TGT GCT CGG TTG TG
RnET1081	ACT GTG TCT CTG CTC TCC GA

The above sequences were designed by identifying regions of loose predicted secondary structure and choosing sequences of 20 base pairs meeting the following criteria;

a) 50-60% G+C content

b) predicted melting temperature of 55-80 °C

c) <4 runs of bases of any kind

Regions of loose secondary structure were defined as sequences of >8 adjacent bases in the mRNA sequence for which there were no predicted intra-molecule Watson-Crick base pairing determined using the Wisconsin Genetics Computer Group software package RNAFOLD.

The mRNA sequence was then imported into the 'primer' package on the Sheffield 'biocomp service' and complemented (G for C, T for A etc.). The regions of loose predicted secondary structure were sought and the oligo criteria were selected. The 3' most bases were aligned with the region of no predicted secondary structure. If the sequence highlighted met all set criteria the sequence and position was recorded. If the sequence failed to reach the criteria the highlighted 20 base sequence was shifted 5' until either a sequence was identified meeting the criteria or the <5 bases remained within the region of loose predicted secondary structure at which point the region was rejected. When more than one 20 base sequence met the criteria at a particular region of loose predicted secondary structure the sequence with the most central homology to that of the loose predicted secondary structure was selected.

Current data relating to the efficacy of ET-1 antisense material is *in vitro* cell culture work. Selected antisense molecules are transfected into cells in culture and the level of ET-1 protein determined by western blotting and/or immunofluoresence using ET-1 antisera. A down regulation of ET-1 protein results when selected antisense molecules are used. Currently we are unable to identify the mechanism by which ET-1 protein is down regulated (ie destabilisation of mRNA, inhibition of translation). Further work will

optimise conditions with respect to providing a suitable composition for use in *in vivo* studies.

The working of the invention is undertaken by using conventional techniques which will therefore not be described herein in great detail. Suffice to say, that the hereindescribed antisense material is manufactured in conventional manner using laboratory techniques and then suspended in a suitable fluid for the purpose of delivery (3). When thus manufactured the antisense material is suitably contained, ideally in a cartridge, which cartridge is adapted for use in a conventional inhaler and more preferably the inhaler described herein (2).

In this way, the invention is worked in a conventional fashion.

1 Delivery of genes using cationic lipid US patent no. 5641662

2 Inhalers. UK patent application no. 9805185.7.

3 Nyce, J.W. and Metzger, W.J. DNA antisense therapy for asthma in an animal model. Nature 285: p721-725. 1997.

CLAIMS

1. A method to treat pulmonary hypertension comprising administering a therapeutic composition to an individual, wherein said composition comprises at least one aerosolised antisense ET-1 molecule, delivered to the lungs by an inhaler during inhalation by a patient.

2. A method according to Claim 1 wherein said composition is delivered to said lungs as a pulse/spike during inhalation.

3. A therapeutic composition for treating pulmonary hypertension comprising a non-coding strand of the ET-1 gene, or fragment thereof, as represented in Figure 1; and, optionally, in combination with a suitable carrier.

4. A therapeutic composition according to Claim 3 wherein said non-coding strand of the ET-1 gene is a oligodeoxynucleotide, or a modified oligodeoxynucleotide, or a oligodeoxyribonucleotide, or a modified oligodeoxyribonucleotide.

5. A therapeutic composition according to Claim 3 or 4 wherein said non-coding strand of the ET-1 gene is between 10-100 nucleotides in length.

6. A therapeutic composition according to Claims 3 or 4 wherein said non-coding strand of the ET-1 gene is greater than 100 nucleotides in length.

7. A therapeutic composition according to Claims 3-6 wherein said oligonucleotide has a G + C content of at least 50%.

8. A therapeutic composition according to Claims 3-7 wherein said oligonucleotide has a G + C content of between 50% and 60%.

9. A therapeutic composition according to Claims 3-8 wherein said oligonucleotide does not contain a consecutive run of more than four bases of any single kind.

10. A therapeutic composition according to Claims 3-9 wherein said oligonucleotide has a predicted melting temperature of at least 55^0 C.

11. A therapeutic composition according to Claims 3-10 wherein said oligonucleotide has a predicted melting temperature of between 55^0 - 80^0C.

12. A therapeutic composition according to Claims 3- 11 wherein said antisense ET-1 molecule is of human origin and comprises at least one from;

i) CAG CCC AAG TGC CCT TTA AC
ii) CTC AAA GCG ATC CTT CAG CC
iii) AGC TCA GCG CCT AAG ACT GC
iv) TGG CAG AAG TAG ACA CAC TC
v) TGG TCT CCG ACC TGG TTT GT
vi) ATG TGC TCG GTT GTG GGT CA

or an oligonucleotide sequence homologous therewith.

13 A therapeutic composition according to Claims 3-12 wherein said antisense ET-1 oligonucleotide is of rat origin and comprises at least one from;

i) ACA GCA GAG AGA AGA TCA CG

ii) TGC ACT TCC TTC TCA GCT CG

iii) GGA TCG CTT AGA CCT AGA AG

iv) CTT GAT GCT GTT GCT GAT GG

v) AGT CAA TGT GCT CGG TTG TG

vi) ACT GTG TCT CTG CTC TCC GA

or an oligonucleotide sequence homologous therewith.

14. A therapeutic composition according to Claims 3- 11 wherein said non- coding strand of the ET-1 gene is adapted to hybridise to the transcriptional promoter region of the ET-1 gene to inhibit transcription of said gene.

15. A therapeutic composition according to Claims 3- 11 wherein said non-coding strand of the ET-1 gene is adapted to hybridise to splice junctions between exons and introns of prepromessenger RNA encoding the ET-1 protein.

16. A method for detemining the efficacy of a therapeutic composition in the treatment of pulmonary hypertension comprising administering a composition according to Claims 3-15 to a selected human or animal and observing the effects of said composition on pulmonary hypertension.

17. An inhaler comprising means for administering the therapeutic composition according to Claims 3-15, wherein said therapeutic composition is administered via a pulse or spike during use of the inhaler.

18. An inhaler according to Claim 17 wherein the inhaler is disposable.

19. An inhaler according to Claim 17 wherein the inhaler is adapted to receive a cartridge containing antisense molecules according to Claims 3- 15.

20. An inhaler according to Claims 17 -19 wherein said inhaler comprises a removable cartridge wherein said therapeutic composition is stored prior to use.

21 An inhaler according to Claims 17-20 wherein said inhaler comprises a rechargeable cartridge in which said therapeutic composition is stored prior to use.

22. A method for administering a therapeutic composition according to Claims 3-15 comprising;

i) adding or providing said therapeutic composition in a suitable dilutant, carrier or excipient and in dosage form to, or in a reservoir of, an inhaler;

ii) aerosolising said composition by suitable means;

iii) detecting the air pressure within the inhaler by suitable sensing device to determine a patient's respiratory cycle; and

iv) delivering a prescribed dose of the therapeutic composition to the patient's lungs during a selected inhalation phase of the patient's respiratory cycle.

22. A kit for treating pulmonary hypertension comprising a therapeutic composition according to Claims 3-15, an inhaler according to Claims 17-21, an air pressure sensing device, and a valve containing pressurised cylinder means to regulate the dosage of said composition.

Figure 1


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   1 GATATCCTAT TAATACAGAG ATACAGAAAG AAATACATAA AAAATAGTTT TATCAAATAC
  61 TTTCCAGCAT TCAAGTGTAG CCTCAAAAGC AAGAATAGGC CAGGAGTGGT GGCTCACGCT
 121 GTAATCCACA GCACTGTGGG AGGCCAAGGT AAGAGGATTG CTTGAGGCCA GGATTTCAAG
 181 ACCAGCCTAG GCAACATAGT GAGATCCCTA TCTCTACGAA AAAATTTTAA AACTTAGCTG
 241 GGCATGGTGC TTGAGCCTGT TGTCCCAGCT ACTCAGGAGG TGAAGTAGGA GTGTCACTTG
 301 AGCCCAGGAG GTTGAGGCTG CAGTGAGCTA TAACTGCACC ACTGCACTCC AGCCTTGGAG
 361 ACAGAGTGAG ACCCTGTCCC CAAAAAAATT AAAATTGAGA AAAAAAAAAA GGCAAGAACA
 421 GCCACAGCAA ACTTTCTATT GGGGAAAAAA AAAAATCCTC CTCTTTACAT CTCTCCCTTC
 481 CTTCCCTTCC CTTTCTGAGA GTGACTGTGG CCAAAAGGAG CATTTTCCCC CTGCAGTCCT
 541 CTGAGGGGTG GGGTGGGGCT ATGAAGCTAT CCTTCATATT CACTCCTTTG TCCAGCTCTT
 601 TTCACCTCTA GTTCTTCTCC CCGCATCTCT GTCTAGCAGT GCCTTAAGTG GAGGAGGGGT
 661 GGGGGCATCA AGCTTGTAAA ACTGGTTTGT TGGGGTTCTC CTTCTCCCCT CATTTCTTGA
 721 TTCTTGGGAA AATGTCTTGC TGGGAGGCTG CCTGGCGAGT GCCCTAGCTG CCTTCTGTGG
 781 GCTTGAATGG GGCTTCCCTC TGCCCCTACA GGAGGAAAAG GGAGCTGCTG CCAGAGGGAG
 841 AAATGGAGAG ATGGACAGAG AAGGCAGGTG CCACCCCTCG CCCCTGACAC ACAAAGAAAA
 901 AGACACGGAA ATTCTCTCTC TCTCTTCTCT TCTCCTATCT CTCTCTCTCT CCCTCTCTCT
 961 CTCTCTCTCT CTCTCTCTCA CACACACACA CACACACACA CACACACACA CAGGCGCGCG
1021 CCGCGCGCGC AGGCACACGT CTTGCAAATT CAGGATTCAA AGAGACAGGG GCACCATTAT
1081 ATTTGGCACG GTGGGGCCTT CCAGGTCTGA AATCCTGCAT TCTTCCTTAC TATTTACTTT
1141 CCCCGAGCTC GAGAAGGGCC AGGTGTGGGC GGATGGCTGG CCACGTTTTG TGTTTCCAAT
1201 TCATATTCAC GGGATGACAC AGACGGGGCG TGGTGAGTGC TGTTGGAGGC GCTTGGGCAG
1261 TTTCATTTTG CCCCACTTCT CCACCTGAAG GCTGGGCGTT GCTGGAACCT GCAGGGGCAG
1321 CCTCAGCAAG GTGGGGTGGC GTGGAGTGGG GTGGGAGAAG GGACTCCAGC TGAAGTAGAA
1381 CCCAGGCTGG ACCTGAGAAT ATTGGGGAGG GCATGGGCGG TGGTTTCCGG GTAGGGGCCT
1441 TGAGGACATG TTGGTCCTGA CTGTTGTCAG TGTTTGGTCA AAGTTGCCAA AAGGTTAAAA
1501 AAAAAAAAGT AGGGGGAGTC CCTGCCAAGA CATATTTCCC AGGCCACCTT TCTTCCGCGG
1561 GAGTGTTGGG GGGGAGGCGC TGCTTGGAAC CTGTGAATGT GACATCAGCT CTCCTCTCCT
1621 CTCCCAAGGT CGGCTTTGGA GAGGGAGGTC AGGGCACCCT TGCCTGGCAC AGGCACGCTG
1681 GCTTCCGGCT CAGTGCCGCC TGCTCTCCGG GAGCTGTGCG CTCCCTGGGC CCCGGGGCTA
1741 GGCTGAGGTA AGCGCACAGC GGAGGCCAGG CGCGCCGGCA GAGGCCTGGG GGATAGGGTG
```

Figure 1-2

1801 GAGGCATCTTC TGGGTGTGTGGG TGTGGGTGTGTG GGTGTGGGAG GGAGAGTTCT TGCCTCTCTTC
1861 TCTCCCATTCT CCAACTCCTTTG CTTCAGTGGC TCTTTTTAGAG GATGCATGTC ATTAATGGACC
1921 TGTCGCTGCC ACTGTCCCCAGC TGTGACTTCG AGGGAGGTCT GGGGATCTGA
1981 GTCTGTCCAA ACCCACGGCT TTGCTGTTGG GTCCTTTTTG TTTTTAGAAGG
2041 AGGAGGGAAA AAAGGTTTCC CAGCATGTGT GTTGTGCCAG TCTTGGAAAT TCATCCGTGC
2101 TTGAATTCCA CCCTCCATCC CCAGAAAAAC TGGAGTTAAAA CAAAAAGAGG AGATGGACAA
2161 AGTGTGTATT TGATGGCATC CCCTGGGGAAG AGACTCTAAA TTTATTCCCAT AGCTCTTACT
2221 GGGCCACTGT GAGCGCTTTG GTGGAGACCA AACAAAATT CTGGGTGCTC AGTTGTCTAA
2281 CCTGAAAAAT GGGACTAGCG AAAAAGCCA ATGTGTTCCA TGCACCTTTT GCTTTCTTTA
2341 TTAAGGCATG ATGTCACCTG TACAGTAACT GCCCTGTGTG TACTTCAGGG GGGGATTTCA
2401 AGCTTAGATA GACAGGAAAT TGTTTTGAAA ATGTAAACAC ATTATTAAAT GTGAACATT
2461 ATCTGATTCC TTGTTCGAAT GGCATTTCCT TCTCAGCACC ACCTTCCTTG CATATTCACT
2521 TAACCCTTGTA CAAGAACACC TTTTTGCCCT AAATGAAGAC ACCCCCCCAA AAAAAGAGT
2581 CCCAGAAAAT ATGTCCCTGC TTGTCGCGGA ATAAATAGAA TATTCTGAGG TGCATTCCTC
2641 CTTCCCTATGT TAGGCAACAT TCCTTGACCC TCCTCGGCCC CCAAGCCAGG TTGGCGTTTTT
2701 TTCTGCCATT TAGAAGGGTT TTCCTTTTTG TCCTAGTAAA ACATCAGCCC CTGTAGCTCT
2761 TCATCTCCCC CTGGTGTTCT TCTCCCGCCA TGTCTTAAGA TTGGTGGCAC CGACCAATCT
2821 TAAGATTTAA GTTCTGTGTG AAAAACACCT TTGCTTTTCA ATCAGTTTAT CAGCCTCCTC
2881 CGCAGGGGAA TGTGGACACA CAAAAGACT TATCGGGGCT TCTCATCAGT GATAGGGAAA
2941 AGACTGGCAT GTGCCTAAAC GAGCTCTGAT GTTATTTTTA AGCTCCCCTTT CTTGCCAATC
3001 CCTCACGGAT CTTTCTCCGA TAGATGCAAA GAACTTCAGC AAAAAAGACC CGCAGGAAGG
3061 GGCTTGAAGA GAAAGTACG TTGATCTGCC AAAATAGTCT GACCCCCAGT AGTGGGCAGT
3121 GACGAGGGAG AGCATTCCCT TGTTTGACTG AGACTAGAAT CGGAGAGAGCA TAAAAGGAAA
3181 ATGAAGCGAG CAACAATTAA AAAAAATTCC CCGCACACAA CAATACAATC TATTTAAACT
3241 GTGGCTCATA CTTTTTCATAC CAATGGTATG ACTTTTTTTC TGGAGTCCCC TCTTCTGATT
3301 CTTGAACTCC GGGGCTGGCA GCTTGCAAAG GGGAAGCGGA CTCCAGCACT GCACGGGCAG
3361 GTTTAGCAAA GGTCTCTAAT GGGTATTTTC TTTTTCTTAG CCCTGCCCCC GAATTCTCAG
3421 ACGGCGGGCG TCTGCTTCTG AAGTTTAGCAG TGATTTCCTT TCGGGCCTGG CTTTATCTCCG
3481 GCTGCACGTT GCCTGTTGGT GACTAATAAC ACAATAACAT TGTCTGGGGC TGGAATAAAG
3541 TCGGAGCTGT TTACCCCCAC TCTAATAGGG GTTCAATATA AAAAGCCGGC AGAGAGCTGT

Figure 1-3


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3601 CCAAGTCAGA CGCGCCTCTG CATCTGCGCC AGGCGAACGG GTCCTGCGCC TCCTGCAGTC
3661 CCAGCTCTCC ACCGCCGCGT GCGCCTGCAG ACGCTCCGCT CGCTGCCTTC TCTCCTGGCA
3721 GGCGCTGCCT TTTCTCCCCG TTAAAGGGCA CTTTGGCTCA AGGATCGCTT AGGATTCGCA
3781 GGAGACCCGA GCGCTTTGAG GGACCTGAAG CTGTTTTTCT TCGTTTTTCT TTGGGTTCAG
3841 TTTGAACGGG AGGTTTTTGA TCCCTTTTTT TCAGAATGGA TTATTTGCTC ATGATTTTCT
3901 CTCTGCTGTT TCTGGCTTGC CAAGGAGCTC CAGAAACAGG TAGGCACGCT CGTTGACTTG
3961 TAAGTCCTCGG AATTACAAGT TAGTGTGTTC TTATCCACCT TCATGCTTTT CTTGCTTCTA
4021 TTTTTCCCCG TTCTTTTTAT GACTGCAGCT TAGAGAGCAA GTGTCTGAGA ATTAATTGCTG
4081 AAACGTACTT TAAGTCTTCT AGTGTAAAAT GTAAAAATCC TCTACTGAAT ACAATTTAGGT
4141 GCAATTGACT ATAACATGAC ATTTAAAATAA CTTATCGTTT TATTATTATT ATTCCATTAT
4201 GTGTTTCCTT GGCTTTTAAA AAATGGAAGA AGTATGGACA TATACAATTT AGTCAAATGT
4261 ATGTTTTGTAA TATTATGTGTT TATACAGGTA CACAGGCCAT ATAGGAACTT AAATCTTATT
4321 TAAACATTAT TTTTAATAGTG TGTTAACGTG TAAAATATTT AAGCATTCCA GCTTTGAAGCC
4381 AAGGAATTGT ATCCAGTCGT TCAAGCAATG TATGTTCAGT AAAATCACCT GCAGAGCAAA
4441 AGTCTGTTGA CTAACTAACG CCTCCCCCGC CCCCCCACCA CCCCCCGCAG GCGGTTTCTG
4501 GGTGAAGCAG ATGTTTTCTT TAAAATTTGT CATCATTGAC TTTTAGTTTC TTTTGGCAGG
4561 TTTTTTGGCAC CCAAAACAGT GTGAGCTCTC TTTTTCAGCTT TATTCACCTG TGCTGGGAGG
4621 GGAGCTAGGA TAATTCTTTG CTGCCGAAGG ATTTAGGCAG TGCGTGTGCA TCTGCCCGGG
4681 TCCCCCCCGT TTTTAGGGTC AGTGCACTTT TTTTGTCTTT TCGTGACCCT GACTAAAGAG
4741 AAAGGATGTC AAGGGAATGA AAATCCTGGA ATTGTGTCTGA TCATTTGAAA TGTACAAAAT
4801 TGGGCAGATA AGCTGCATGG CTAAATTCTT AGGAGAGAGA GGCAAGGCAG TAGTGGAGAA
4861 GGGGGAGGCA GTGGATCCCA CACAAGCCTG ATGCCCCAGGG ATTCGGAATT CAAAATCCCC
4921 CCAGCCTACC TTCAGTCCCC TGACCTGCTT CTCAGCCCCA CCTTAGGTCA CTGGTTTCTA
4981 TGGAGTTACC CTACTGAATT GAATATTGAA TAGTTAATTT CTCTCTCCAA TCATTTTCCC
5041 CACCTAATTT TGAAAGATAT ACATCATCTG GGGTACCCTG TGCCCTACAC AGCATGTGAA
5101 GTGGATGGGT ACCCCCTAAA GAGAGGTCA TCCTGAATGG GGAAGTGGCC CCAAAGCTAG
5161 GAATAACTGT GATTTCTTGT CTTTAGTCAT GTGCCAATGT TAAGTAAGCT TACAGTGGATA
5221 GTGCTGTCCT ACCAAGTTCC TTGTAGAAGC CAGCCGGATT TTCAACACGC AGCATTCCAC
5281 AGCATTTCCC TGAGCCTGCT TCAAGAGGGG TGGGGGAAGT CCCTTCTTGA GTCGTTTATCT
5341 CCTCTGCATT TGTGTAATCT CCCTGAAGGT GGATAAGCCA AGGGCATGAG GGGGAGGCAA
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Figure 1-4


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5401 AAGGTGAACT CATGTTAAGG AGGGAAAAAA ATAAAGAGCC CTTTTTTCTG TGTTTCTTGC
5461 TGATGGCAGG CTGTCGTGCT TCATCTGCTTT TATCTGCTCT GCTAGCTCCG ACTCTACTGT
5521 GATCCAGCAT GTCTCCGGC GTTTGAGGAG AGATCCCCCA CTGACCTGCT CTTTCTCTCC
5581 CCAGCAGTCT TAGGCGCTGA GCTCAGCGCG GTGGGTGAGA ACGGCGGGGA GAAACCCACT
5641 CCCAGTCCAC CCTGGCGGCT CCGCCGGTCC AAGCGCTGCT CCTGCTCGTC CCTGATGGAT
5701 AAAGAGTGTG TCTACTTCTG CCACCTGGAC ATCATTTGGG TCAACACTCC CGAGTAAGTC
5761 TCTAGAGGGC ATTGTATACC TATTCATTCA TTAGCCCTGG CTCCACTGGA GCCCAGTTTT
5821 AGAGTTTCTT TTCTAGGGAC TCTGAAGGTA GTCCTTCTAA CACCATCCAA GTGCCTCAGT
5881 GGGGACAGTT TCCCTCTATT CCTGAAAATA ACGACAGCTT CGTTCTTAGC AACCAAGGGG
5941 AGGGTCTTCT GAGGCCCCGT AGCTCAGGCT ACTCATGATG GGACAAGCAG GAGGCCACTG
6001 CACGTTTCAA ATGAGGAACT TTCAGTGAGA GGGCCTCAGG GGGACACTCT CACAGTGGCA
6061 TCTGATGGGG TTTCGGGAAT AATTGCCGAG GTCAGATGTG GGTTAGTGCA ACCTGTGCTT
6121 CTCATGGGAG GGTGGAGACT GAGAGGCAGA AGTGATGATA TAGAGGGTTA GAATCACTTA
6181 ATTTTAGTTA GCAACTAAAC TAGGCTCAAA GTCATTGTGA AGCACTTTGT CGAGTTGAGT
6241 TGTAGCAGAG CTAGAACTGG AGCCCGGATT TCCTTTGCTG CTATATTTTC CCTTTAGAAA
6301 TGCCCATTTC AGAACTGAAA TAGAAATACT GTCCAATGGC TTCTCTTTCA CCTACAGAGA
6361 AGAAAAGCAG ATTTCCCTCT TCTGCCCTGG ACACTAGTTC ATCATCTGTC GGAAGCAGTC
6421 ATAAACAAGC ACACATTTAC TATGCATACA ATGTACCGTT ATGACAAAGG AGGACCAAAA
6481 TCCAAACAAT ATCAAACCAC ACCAAAAACC ACAAGGAGCC TAATAATTAC TAAGGTGATA
6541 CTTCCAAAGG GAGGACTTTA TTTCTTAGAT GAGAATGAAA ATGGACACAT TGGAAATTAT
6601 TGGAGAGCCC TCTGGCTATG AGTCCTTCCA CAACCATATG GTACCACCGA CTGGCAGGAG
6661 AAATGTGTGA ACATGTGCCT CCTCTCCCCA ACCACTGGGG CCGGTGGGGT GACGGTGGCA
6721 CTTTTTAGCAG TATCCTCCGT GGTTTGAGTT GAAAATAAGT TTTTAAAAATC CTCTGAGTCA
6781 TGGTTTTGCA TTGAAAACCTC TTCCCACTGT GTACCCACAA ATAGTTGTGCA GGAGTGAGTT
6841 TTTAGAAAAGG AAGAAAATAT AAAGCAGATG CCAAGCAGAG ATGTCCCTAT TTTTGACAAA
6901 AAAGCAATGT TGCTTTGTGC AAGAAGAAAC TGAACTTTGT GAAGAGTTGA AATGGAATTC
6961 CACTGAATTA GAAAAACTTG TTTTCTCCTG CCTGGATACA TACAGTCAAG GCCATTGATG
7021 CACAGGTGTT CCTGGCTGTT GTTACACTTT ACCCTCTGAA ATGATGCTCC CAAGTGCTAT
7081 GTGATGAGCT CCTTGTGTGC CCAGTGGAAT AGGTGTGTCC ATGTGTCATT TTAAAGACTA
7141 TTAATATAGTT TCTTTCTCTC TTTGGATAAT AGGCACGTTG TTCCGTATGG
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Figure 1-5


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7201 ACTTCGGAAGC CCTAGGTCCA AGAGAGCCCTT GGAGAATTTA CTTCCCACAA AGGCAACAGA
7261 CCCTCAGAGAAT AGATCGCCAAT GTGCTAGCCA AAAAGACAAG AACTGCTGCA ATTTTTGCCA
7321 AGCAGGAAAA GAACTCAGGT GAGCAGAAAC ACCTTTGCTT TTCAATCAGT TTAACAGCCT
7381 CCTGAACTCC TTCCTATCAT GGTACTGCCT TCCTGTTTTA GAGAGACTAA CAAGACACTT
7441 GAAAGTCAGG GTAAAAGCTGA ATATAACATT GCTGAAATGT TTTTTCCTTGT GTAATTTTAAC
7501 AGGGCTGAAG ACATTATGGA GAAAGCATGG AATTATCATA AGAAAGGAAA AGACTGTTCC
7561 AAGCTTGGGA AAAACGTGTAT TTATCAGCAG TTAGTGAGAG GAAGAAAAAT CAGAAGAAGT
7621 TCAGAGGAAC ACCTAAGACA AACCAGGTAA GAGGGAAGGA AGAAAAATTA GGTAAGAGGT
7681 TCACAAGAAC AACTAGCCCC AGTCAGTGAT GCCAGAGAGC TGTTCCTCCA GCCCTTCTTA
7741 CCCGGGGCAGG TGAAAGACTT AGAAAACAGT AGCAGAGGAG ATCTATATGCAT CCTATAGATT
7801 AAAAGGAGCA AAAGAATCCC TCTTAAATAT TTCCAATGAAG CTCTGGAAATG CAAACGGATG
7861 TCCTCTGTAC CTTTAGCACA TACCATTTCA TCTACAGGTA GATTTCCCAA CCAAAATATA
7921 TCCAGAGATG CCTTTGTCAT TGGGTTATAT ACAGCCTTTG CCTCTCTGAG TCAATGTATT
7981 TACCACTTTC CCTGAGAAAT CCTGAAATCA TTTGGGGAGC GGACATTTAG ACAAAAGAATC
8041 AAAGTGTCAT GGATAATCAA ATTCTTTCAAT AAGTTGCAGT TATTCAGATG GCCAAAGGAA
8101 AAATAAAGTC ATTGAGTAGG GTTGGTAGAA TTTAGAACAT GCTGTTTTTC AGGTTTATGG
8161 TCTTTTTTTTT TTTTTTTTAA TAGGGAAATG TGTTTGGTGC AGAGCCAATG
8221 TCATTCCAAA AAGCTCTCTC TTTTCCCTGGT CAGTCATGTG CTGGGACAGA GAAGGGATCT
8281 GGATTAGGCA ACATCATAGA GTTGCTCTGA GCTGCTCTTT GGTGATAACC CTTTCCAAATC
8341 CTAAACTTTT TGGAATTCAC AAGCTCAAAG GAGGAAGCCT ACTCTCTGAT CTACCACATG
8401 TTCTGCATTT TTCTATCATG GTCTATGGAA ACTTCTCCTA GAAATCCAGT GGCAAGAAGT
8461 TTCTATGATTA AAGTGTCTG AGCTCAGGCC AGGCAGTCAT GAACTACTTC TGAGTTGTTT
8521 ACTACTGATT TGTGGGGCAG CCTCAGCTAT CGGTTTCTTC ACACCTGCTT ATGAGAGTAT
8581 CCATATTTAT GGTCGCAGGC AGTAATGCTC CCCAGGAGAT CAGTTTCTGA ACTAACCTGG
8641 AATTTTTTAT GGGTTTTTAT TATGCCAACT ATTAAATCAA CATTACAGTT CTTCCCTCTG
8701 TATTTCTCCT GTAAAACATT AGGCCTGCAA AAAAAAAAAA TCTTTTTAAA AATTATTGCC
8761 ATTAAAGTATT TGCTCTGGGC CTTACTGTATG CTTCTTTTYT TTTTCTCTCT TTTTCAACTAA
8821 GTCACCGTCA ATTTATTAAG ATGGCCATAA CTTATTCAAAA CCTATGCTGA GTTCCCTCAAG
8881 GCAGGGTCGC ATAGTGATGA AGGTTGGGAT GGGGCTAGCG AAGAAACCAG AACTACTCTA
8941 GTTTATTTTAA AACCTGTATT TACTGCCCAC TTCCCCTTAG ACTTGACCAT ATGACCCTT
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Figure 1-6


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9001 GCTCCCCCATTT CTAAGCATAG GGGCAGGCTT TATTTTTTACA ATGGTAAATAG ATGATATCAC
9061 TTGAGGTTTTT ATCAAAGAAGT TGCGGGCGGGT GGTGAAAGTT CACAACCAGA TTCAGGTTTTT
9121 GTTTCTGCCA GATTCTAATT TTACATGTTT CTTTTTCCCAA AGGGTGATTT TTTTTAAATA
9181 ACATTTTGTTT TCTCTTTATCT TGCTTTTATTA GGTCGGAGAC CATGAGAAAC AGCCGTCAAT
9241 CATCTTTTTCA TGATCCCAAG CTGAAAGGCA AGCCCTCCAG AGAGCGTTAT GTGACCCCACA
9301 ACCGAGCACA TTGGTGACAG ACCTTCGGGG CCTGTCTGAA GCCATAGCCT CCACGGAGAG
9361 CCCTCTGTGCC GACTCTGCAC TCTCCACCCT GGCTGGGATC AGAGCAGGAG CATCCTCTGC
9421 TGGTTCCTGA CTGGCAAAGG ACCAGCGTCC TCGTTCAAAA CATTCCAAGA AAGGTTTAGG
9481 AGTTCCCCCA ACCATCTTCA CTGGCTTCCA TCAGTGGTAA CTGCTTTGGT CTCTTTCTTC
9541 ATCTGGGGAT GACAATGGAC CTCTCAGCAG AAACACACAG TCACATTCCA ATTCGGGTGG
9601 CATCCTCCCG AGAGAGAGAG AGGAAGGAGA TTCCACACAG GGGTCGAGTT TCTGACGAAG
9661 GTCCTAAGGG AGTGTTTGTG TCTGACTCAG GCGCCTGGCA CATTTCAGGG AGAAACTCCA
9721 AAGTCCCACAC AAAGATTTTC TAAGGAATGC ACAAATTGAA AACACACTCA AAAGACAAAC
9781 ATGCAAAGTGT AGAAAAAAAA AAGAAAGACT TTTGTTTAAA TTTGTAAAAT GCAAAACTCA
9841 ATGAAACTGT TACTACCATA AATCAGGATA TGTTTTCATGA ATAATGAGTCT ACCTCACCTA
9901 TATTGCACTC TGGCAGAAGT ATTTCCCACA TTTTAATTATT GCCTCCCCAA ACTCTTTCCA
9961 CCCCTGCTGC CCCTTCCTCC ATCCCCCATA CTAAATCCTA GCCTCCGTAGA AGTCTGGTCT
10021 AATGTGTCAG CAGTAGATAT AATTATTTTCA TGGTAATCTA CTAGCTCTGA TCCATAAGAA
10081 AAAAAAGATC ATTTAAATCAG GAGATTCCCT GTCCTTGATT TTTGGAGACA CAATGGTATA
10141 GGGTTGTTTTA TGAAAATATAT TGAAAAGTAA GTCGTTTGTTA CGCTTTTAAAG CACTAAAATT
10201 ATTTTTCCTTT ATAATAACCGG CTAATGAAAG AGGTTGGATT GAATTTTTGAT GTACTTATTT
10261 TTTTATAGAT ATTTATATTC AAACAATTTA TTCCTTATAT TTACCATGTT AAATATCTGT
10321 TTGGGCAGGC CATATTGGTC TATGTATTTTT TAAAATATGT ATTTTCTAAAT GAAATTGAGA
10381 ACATCGCTTTG TTTTTGCCTGT CAAGGTAATG ACTTTTAGAAA ATAAATATTTT TTTTTCCTTAC
10441 TGTACTGATT TGGAATCATT ACTTGAAATTT GTAAGGAGTG GGCCAACGTG AGTTAAGTACC
10501 ATAAAGGCAA ATAAATGGTT AAAGACGGTT TCATAGAAAA GTGACAATTA GAAGATATT
10561 ACCGCTCTAAG CTAATTATAT AAAGAATTTT ATCTCTATCT TAAATGTTGA TTTTATATCTG
10621 CATTGAGGTA AAAACACAAA ACAAAAAAGC AGCTTTTAACA CCTCTGTCCT CTCTTGGGTA
10681 GCAGCCTCCT GCTTCTCCTT CACCTGAAAA ATTCTCCAGG GACTTCATCC ATTTAACTGG
10741 CTCAGGCTAT TGGCAGGATT CACAGTTTAA GCTGATGGTG TGGTGAGAGA TGCTTTATCC
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Figure 1-7

10801 ATATTTAATGG ACTGAAGGAA GTAAATGGCAA GACAACCCCC CAAAACATAC CTAAATTATAC
10861 AAAGTTATTAT ACCAAAGTTG CTTTTTAGAAA ATGGCCCTGCT CAGAGCAAGT AGAGGTTTCC
10921 AATGGCTTTTT TATTTTCTCA CATTTAAGGAT GTTGTTTCTT AAGGAACATT GAGTACCATT
10981 GCTTTCTTCGT GATAGCCTAG GACTGCCGTG TGCCCATGGA GGTAGAGACA CCAGGTACTG
11041 ATTCTTAGGTC CTCTGCCACA AAGCACCACT TCCTCTCCAC TTTGCCTTGG CTGGCCCTGT
11101 CAGCTCACTG GAGAGCACAG TATTTGCAATT GCAGTATTGC AAATGGTCAC TACTTACTGA
11161 ATTCTCTAAG AGCTTGATTA GCCCTCGAGA ATCTTCCTTG CCCTTCTCTA ATAGTGTCTG
11221 AAGGAATTCC TGGCATTTAA CAAATATTAG CATGTAGTCA TCACTCGTCG TCCTAACAGTG
11281 ACACATCAGA AGGATTTCAA ATAACAGTCT TCAGGCATGC GTAATCAATG TCCTGTGCAG
11341 AGTCTCCGTC CTCATTGATC CTCATTTTTC TCTTTAAGGC ACAGTCCAAT GTCCTTTGGG
11401 AATTCTTTAT AAAGCTTACT TTATCCATAA ACTGTTTCTC AGTGCGTGAC TCTGAAGAAA
11461 ATTTTCAAGT TTTGCCCATG TTGACAAGCT GCTTGGTCTG AACTTGGCCA GTATTTAATC
11521 TTGAGCAAAC GATTCAATTT CCTTCTATCG TGAGTTTTCT CATCTATGAA ACAAGGGAGT
11581 TGAGGGGAGT TTCTTTCATA CCTCTGAGAA AGAGTTTGAG ATTTACATAAA GAAGTTGAAG
11641 TGGCATGAAA AAAAATAAAG ATCTGAGGCTT AGAAGACATG GATCTAATAC ATTTTAAGAGG
11701 AAGTCAGAAT CAGAGAAGCC ACTGAACAAA ACAGTCCAAA CGGAGCATAG CAGTCAGAAT
11761 TGATGAGTTT TGGTTGGGTT TTTCATCAGT CAAACCCTTG AGCCCCCCTT TCCCATGCTT
11821 CCTGCTTCAG TATCCAGTAG GAAAAATGAA AGGGATGATG TAGACACTCT AGGGCATGAG
11881 GATTTGCAGT AAATAAGTTG GGAGACTCAC AGAAAATTAA TATTTTTCAA ACATGAAGAC
11941 GAAACATTTCA ATTATATTAC AGTCCACATC AGCTTGAAGG GTAAACTGAT GGGATGATCT
12001 GTCACATTTC TTGCTTCTGT TCCAGTAAAA GCATGGTTTC TGGAAACCCA CTTAGGACAG
12061 CTTTCTCTCCT TTACACTGAT AGCCCAGGCA AGCTTTGATC TCAGAACTCC AGAAACCAGA
12121 GAACTCTAGG TGGAATGTGG TAACTTTTTGC CAGGGCAGAG GGAACACCTA CTAATAGGTA
12181 CTTCATTTTGC ACCACCAGAG ATTGGCATCT TTTTTGATGG ATCCACTGGC TTTGATACTG
12241 CCTGTACTCC CCCAAACAC AGCTTGGGTA TTGGACTAAT CTAGAGCTCC CTCAGGAGAA
12301 CTCTTGCTGA CATTAAGAAA GAGCAACATT TTGTCTTTCC AGGTGAAAAT CCAAGGCCAA
12361 AAAGGGAGTG ACTCACCTAA GATCACAGAA GGAGCTGTAG CATCTCTGGA GCCTGAACAC
12421 TTAAGTTAAG CACGACTATT TCACGCAGAG GGCATGAATT C

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 98/02584

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/11 A61K31/70 A61M15/00 A61M11/00 //B65D83/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07H A61K A61M C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SAKAI S ET AL: "Pulmonary hypertension caused by congestive heart failure is ameliorated by long-term application of an endothelin receptor antagonist : Increased expression of endothelin -1 messenger ribonucleic acid and endothelin -1-like immunoreactivity in the lung in congestive heart failure in rats." JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY, (1996 NOV 15) 28 (6) 1580-8., XP002092833 see the whole document --- -/--	1,3-11, 16

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 February 1999	24/02/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Andres, S

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 96 40162 A (UNIV EAST CAROLINA ;NYCE JONATHAN W (US); METZGER W JAMES (US)) 19 December 1996 see page 7, line 2 see page 8, line 23 - page 10, line 11 see page 11, line 17 - line 27 see page 38, line 20 - page 39, line 35 see claims	1,3-11, 16
X	GB 2 283 179 A (HIGENBOTTAM TIMOTHY WILLIAM) 3 May 1995 see the whole document	17-21
X	WO 94 27664 A (KEANEY NIALL) 8 December 1994 see page 5, line 8 - page 7, line 18 see claims	17-21
A	BUTT A.Y. ET AL: "Pathophysiological basis of the treatment of pulmonary hypertension." EUROPEAN RESPIRATORY REVIEW, (1995) 5/29 (248-251)., XP002092834 see page 249, right-hand column, paragraph 2 - page 250, paragraph 1	1
A	STELZNER, T. ET AL.: "Increased lung endothelin-1 production in rats with idiopathic pulmonary hypertension" AMER. J. PHYSIOL., vol. 262, May 1992, pages L614-L620, XP002092835 see the whole document	1
P,X	GB 2 320 900 A (UNIV SHEFFIELD) 8 July 1998 see abstract see page 3, line 18 - page 5, line 5 see claims	17-21

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 98/ 02584

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 1-2, 16 and 22 is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-15,22
   Antisense oligonucleotides against endothelin-1 for the treatment of pulmonary hypertension.

2. Claims: 16
   A method of determining the efficiacy of a therapeutic composition in the treatment of pulmonary hypertension.

3. Claims: 17-21
   An inhaler.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter onal Application No
PCT/GB 98/02584

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